Prevention and reversal of atrial cardiomyocyte remodeling in Atrial Fibrillation

Hu, Xu

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Chapter 2

Keeping up the balance: Role of HDACs in cardiac proteostasis and therapeutic implications for atrial fibrillation

Deli Zhang[1], Xu Hu[1], Robert H. Henning[1], Bianca J.J.M. Brundel [1,2#]

[1] Department of Clinical Pharmacy and Pharmacology, University Medical Centre Groningen, University of Groningen, The Netherlands.

[2] Department of Physiology, Institute for Cardiovascular Research, VU University Medical Center, The Netherlands

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Abstract

Cardiomyocytes are long-lived post-mitotic cells with limited regenerative capacity. A proper cardiac function critically depends on the maintenance of a healthy proteostasis of protein expression, folding, assembly, trafficking, function and degradation, together commonly referred to as proteostasis. Impairment of proteostasis has a prominent role in the pathophysiology of aging-related neurodegenerative diseases, including Huntington's, Parkinson' and Alzheimer's disease. Emerging evidence reveals also a role for impaired proteostasis in the pathophysiology of common human cardiac diseases, such as cardiac hypertrophy, dilated and ischaemic cardiomyopathies, and AF. Histone deacetylases (HDACs) have been recently recognized as key modulators which control cardiac proteostasis by deacetylating various proteins. By deacetylating chromatin proteins, including histones, HDACs modulate epigenetic regulation of pathological gene expression. Also, HDACs exert a broad range of functions other than the nucleus by deacetylating structural and contractile proteins. The cytosolic actions of HDACs result in changed protein function through post-translational modifications and/or modulation of their degradation. Chapter 2 gives an overview of the mechanisms underlying the derailment of proteostasis in AF and subsequently focus on the role of HDACs herein. In addition, the therapeutic potential of HDAC inhibition to maintain a healthy proteostasis resulting a delay on AF onset and progression is discussed.

Keywords: Atrial Fribrillation, Histone deacetylases, Proteostasis, HDAC6

1.Introduction

1.1 Proteostasis

The proper function of cells depends critically on the maintenance of a healthy homeostasis of protein expression, folding, assembly, trafficking and degradation, together commonly referred to as proteostasis.[1, 2] A proper proteostasis enables healthy cell and organismal development.[1] The requirement of maintenance of proteostasis is of importance, especially to long-lived post-mitotic cells, such as cardiomyocytes, that display limited regenerative capacity.[3, 4] Derailment of proteostasis plays a prominent role in the pathophysiology of ageing-related neurodegenerative diseases, including Huntington's disease, Parkinson's disease, and Alzheimer's disease.[4] The impairment of proteostasis is recently also implicated in the pathophysiology of common human cardiac diseases, including pathologic cardiac hypertrophy, dilated and ischemic cardiomyopathies and atrial fibrillation (AF). [3-6]

1.2 Remodeling in atrial fibrillation

AF is the most common progressive clinical tachyarrhythmia.[7] Its incidence is age-related and increasing in the ageing population. AF can be caused by underlying cardiovascular conditions, including hypertension, cardiac surgery, pericarditis, congestive heart failure,[8, 9] but clinical signs of underlying heart diseases are absent in about 30% of AF patients. However, additional risk factors are linked to this group of 'lone' AF patients, including alcohol abuse, obesity, metabolic syndrome, psychological stress and genetic factors.[10-12] AF-associated risk factors, but also AF itself, result in atrial arrythmogenic remodeling, which is central to AF progression and defined as any change in atrial structure or function that promotes atrial arrhythmia.[13] During the past decades, various mechanisms have been identified which promote the occurrence or maintenance of AF, including electrical remodeling and structural remodeling.[14] Compared to electrical remodeling, structural remodeling is perceived as the main contributor to initiation and persistence of AF and may be present before the first episode of AF due to associated underlying diseases.[5] Atrial structural remodeling, as observed in animal models and clinical AF, includes hypertrophy, dedifferentiation of cardiomyocytes and fibrosis. In addition, at the subcellular level, loss of contractile apparatus (myolysis) and changes in size and shape of the mitochondria, disruption of the sarcoplasmatic reticulum and homogeneous distribution of nuclear heterochromatin are found.[15-19] Although structural remodeling induces the vulnerability for AF, the molecular mechanisms underlying remodeling are not completely resolved.

1.3 Role of HDACs in AF associated derailment of proteostasis

Recent mechanistic studies show that the progressive derailment of proteostasis creates a substrate for the initiation of AF, with histone deacetylases (HDACs) playing a central role (Figure 1).[3, 5, 16, 18, 20-24] HDACs have recently been recognized as key modulators in controlling cardiac proteostasis by changing acetylation status of various proteins.[25] By modifying chromatin they modulate epigenetic regulation of pathological gene expression. In addition, HDACs also exert a broad range of functions outside the nucleus by deacetylating structural and contractile proteins, leading to changes in protein function through post translational modifications (PTMs) and/or modulation of their degradation.[6, 26-

[28] Studies from the past decade indicate that HDACs alter proteostasis in cardiovascular diseases, including cardiac hypertrophy and heart failure, mainly by epigenetic regulation of pathological gene expression.[29-35] In AF, however, involvement of HDACs in derailment of proteostasis seems much more associated with changes in PTM of contractile and structural proteins and the degradation of proteins.[6, 28, 36] In the current review, we will summarize key mechanisms involved in the derailment of proteostasis and the modulating role of HDACs in AF progression. In addition, the potential of HDAC inhibition as a novel therapeutic target to treat clinical AF is discussed.

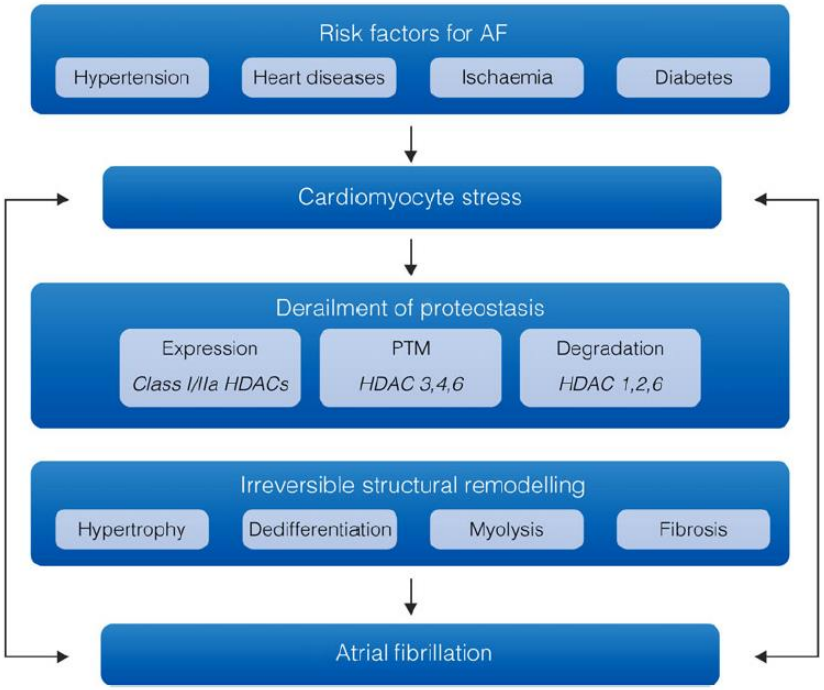

Figure 1: Overview of derailed proteostasis and the role of HDAC in AF progression. Risk factors for AF, such as hypertension, heart diseases, ischemia and diabetes, induce cardiac stress which results in derailment of proteostasis by impairment of protein expression, post translational modifications and degradation. Derailment of proteostasis is regulated by various HDACs, as indicated, and triggers structural remodeling in AF, such as cellular hypertrophy, dedifferentiation, myolysis and fibrosis. These structural changes create a substrate for further AF progression.

2.Central mechanisms of derailed proteostasis in AF

Recent studies revealed that three mechanisms importantly derail cardiomyocyte proteostasis in AF. These mechanisms include changes in protein expression, PTM and protein degradation.

2.1 Changes in protein expression by transcriptional reprogramming

Various studies show that AF induces changes in protein expression, which will challenge the proteostasis of cardiomyocytes (Figure 1). Changed levels in protein expression are caused at the gene transcriptional level by transcriptional reprogramming. A recent study of the atrial transcriptome in patients with AF identified a decreased expression of targets of several transcription factors related to inflammation, oxidative and cellular stress responses and changes in ion channel expression, indicating substantial transcriptional reprogramming in clinical AF.[37] Various additional studies in

patients with persistent AF and in tachypaced dogs, document changes in expression of proteins involved in calcium signaling, such as reduced L-type calcium channel alpha 1 (Cav1.2) and sarcoplasmic reticulum (SR) Ca^{2+}-ATPase expression[38] and increased sodium calcium exchanger expression (NCX1) which was found to enhancing the Ca^{2+} leak from SR, resulting in promotion of clinical AF.[39]

In addition, dedifferentiation of the atrial cardiomyocytes shifts the gene expression profile towards a more fetal phenotype as observed in both experimental and clinical AF.[17, 40, 41] Dedifferentiation includes re-expression of β-myosin heavy chain (MHC), smooth muscle α-actin (α-SMA).[41-45] Together, these data show that the protein expression profile of cardiomyocytes is affected in AF, both by the regulation of specific genes and by a more general transcriptional reprogramming.

2.2 Alterations in post translational modifications

PTMs of proteins are critically important in the regulation of handling of proteins by the cardiomyocyte and thus determine the fitness of proteostasis. PTM refers to the covalent and generally enzymatic modification of proteins during or after protein biosynthesis.[46] Proteins are synthesized by ribosomes, where mRNA is translated into polypeptide chains, which may subsequently undergo PTM to form the mature protein product. PTMs, including (de)phosphorylation and (de)acetylation, alter the function of proteins and are important components of cell signaling.[47]

Acetylation and deacetylation of proteins play an important role in pathological cardiac remodeling and are involved in cardiac diseases, including heart failure and AF.[25, 28-36, 48-50] Protein phosphorylation has been studied in detail in AF and is recognized to underlie AF promotion in experimental and clinical AF.[14, 51-54] Also protein acetylation is found to play an important role in AF progression. In particular, deacetylation of α-tubulin contributes to the development of a substrate for AF progression.[6] Besides α-tubulin deacetylation, protein acetylation regulates fibrosis formation, electrical activation (via connexin 40) and calcium handling and thereby promotes atrial arrhythmogenesis and AF inducibility.[24, 55] Thus, in addition to protein phosphorylation, the protein acetylation status now emerges as a key contributor to AF initiation and progression (Figure 1).

2.3 Derailment by protein degradation

By employing a goat model, Ausma et al. were the first to show that AF is associated with the degradation of the sarcomeric structure, named myolysis.[56] Later studies confirmed that myolysis is a general characteristic of atrial structural remodeling in patients with persistent AF.[16, 18] There are indications that myolysis is caused by persistent activation of proteases, especially calpain 1.[6, 16, 21, 57]

In addition to protease activation, activation of auxiliary cellular protein degradation pathways, such as autophagy may drive AF progression. Autophagy is a pathway removing damaged or long-lived proteins and organelles. Controlled autophagy during (mild) cardiac stress conditions, such as nutrient deprivation, hypoxia and oxidative stress, supports cardiomyocyte survival. In contrast, stress-induced, excessive activation of autophagy causes derailment of cell proteostasis by degrading essential proteins and organelles, thereby triggering autophagic cell damage and death as found in mitral valve regurgitation and cardiac hypertrophy.[58, 59] Interestingly, both cardiac conditions often trigger AF onset.[60] Ongoing research from our lab indicates that autophagy indeed contributes to the initiation and progression of AF.[61]

The findings indicate that derailment of proteostasis via changes in protein expression, PTM and degradation contributes importantly to AF progression. Interestingly, in the past years, HDACs emerge as a key player in cell homeostasis, as they control proteostasis by acting on both chromatin-related and non-chromatin-related pathways.

3.Role of HDACs in cardiac proteostasis

3.1 General role of HDACs

The fundamental processes of proteostasis are all subject to regulation by HDACs, including protein expression, proper PTM, trafficking and clearance. In total, 18 mammalian HDACs are encoded by distinct genes and grouped into four classes on the basis of similarity to yeast transcriptional repressors (Figure 2).[62] Class I HDACs (HDACs 1, 2, 3 and 8) are related to yeast reduced potassium dependency-3 (Rpd3), class II HDACs (HDACs 4, 5, 6, 7, 9 and 10) to yeast histone deacetylase 1 (Hda1), and class III HDACs (sirtuin 1–7), usually named as sirtuins, to yeast Sir2. Class II HDACs are further divided into two subclasses, IIa (HDACs 4, 5, 7 and 9) and IIb (HDACs 6 and 10). Finally, HDAC11 lacks homology with yeast HDAC enzymes and belongs to a fourth class. Classes I, II and IV HDACs have a highly conserved zinc-dependent deacetylase domain and are referred as classical HDACs (Figure 2). The classical HDACs show also high similarity in structure, enzymatic function, subcellular localization and expression pattern. In contrast, class III HDACs (sirtuins) utilize nicotinamide adenine dinucleotide (NAD^+) as a co-factor for catalytic activity and are because of this mechanism associated with aging and various cardiac diseases including metabolic and hypertrophic heart diseases.[63-65] Since this review is focused on the classical HDACs (class I, II and IV, Figure 2), which are effective targets of the commonly used HDAC inhibitors, we excluded class III HDACs from this review.

In general, HDACs are enzymes that catalyze the removal of acetyl-groups from lysine residues from both nucleosomal histone tails and various non-histone proteins, thereby altering gene expression and cell function, respecively.[66] Exceptions are the nuclear IIa HDACs, especially HDAC4 and 5, which are abundant in the heart. Under normal conditions, class IIa HDACs bind to transcription factors, such as myocyte enhancer factor 2 (MEF2), and thereby repress their activity and downstream pathological fetal gene expression (Figure 3). In response to stress signals, class IIa HDACs are phosphorylated and undergo nuclear export, resulting in derepression of the downstream pathological fetal genes.[34] Class IIa HDACs regulate gene expression through recruitment of class I HDACs and by modulation of various transcription factors, including MEF2 and serum response factor (SRF) among others, resulting in cardiac hypertrophy and fibrosis (Table 1). [24, 67-76]

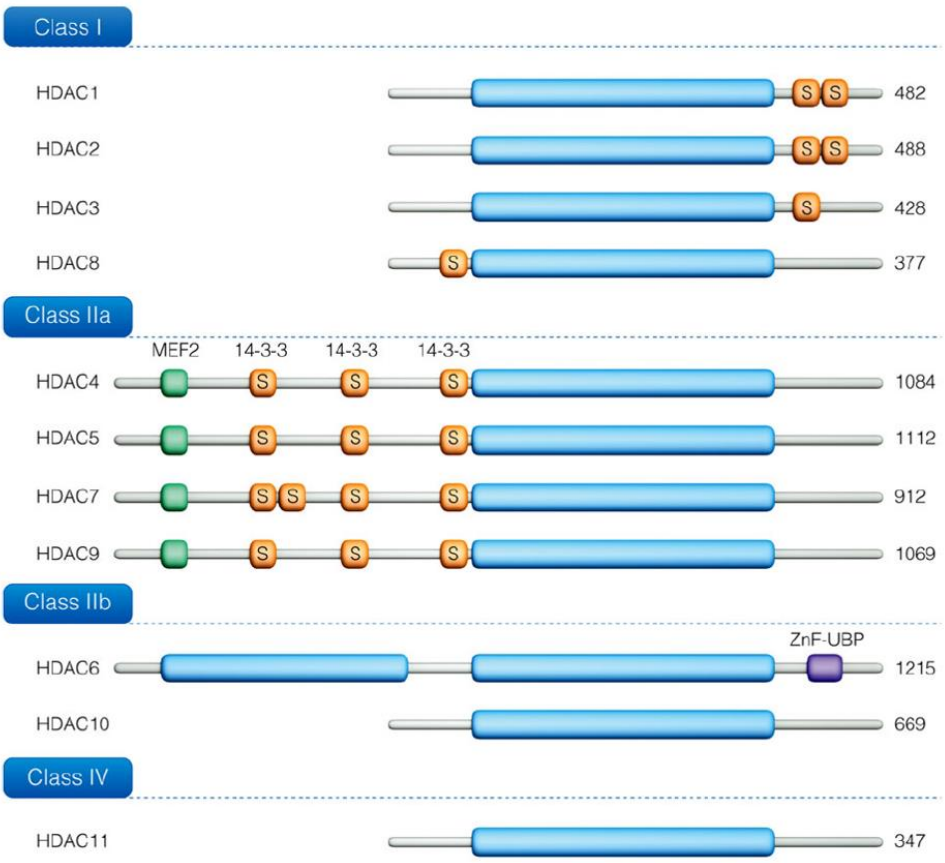

Figure 2: Overview of the classical zinc dependent histone deacetylase (HDAC) superfamily. The classical HDAC family consists of members of the class I, IIa, IIb and IV HDACs. Black rectangles indicate the conserved HDAC catalytic domain. Numbers following the HDAC domain indicate the number of amino acids in human. MEF2-binding sites and binding sites for the 14-3-3 chaperone protein sites are marked by grey squares. S represents the serine residue which can be phosphorylated by kinases. HDAC6 has a zinc-finger ubiquitin-specific protease (ZnF-UBP) domain, which can bind to ubiquitin and plays an important role in autophagy. (Figure is modified from Haberland et al. Nat Rev Genet. 2009.)[66]

Table 1: Overview of cardiac transcription factors (TGs) regulated by HDACs

TF	HDACs	Pathological pathways	References
MEF2	HDAC4 HDAC5 HDAC7 HDAC9	Heart failure, hypertrophy	63, 67, 68, 69
KLF4	HDAC2	Cardiac hypertrophy	70
YY1	HDAC2 HDAC4 HDAC 5	hypertrophy	71, 72
NKX 2.5	HDAC5	Heart development, NCX1 expression	73
Myocardin/ SRF	HDAC5	Differentiation of smooth muscle cells, hypertrophic arrhythmia	24, 74
NF-κB	HDAC1 HDAC5	Cardiac infarction	75

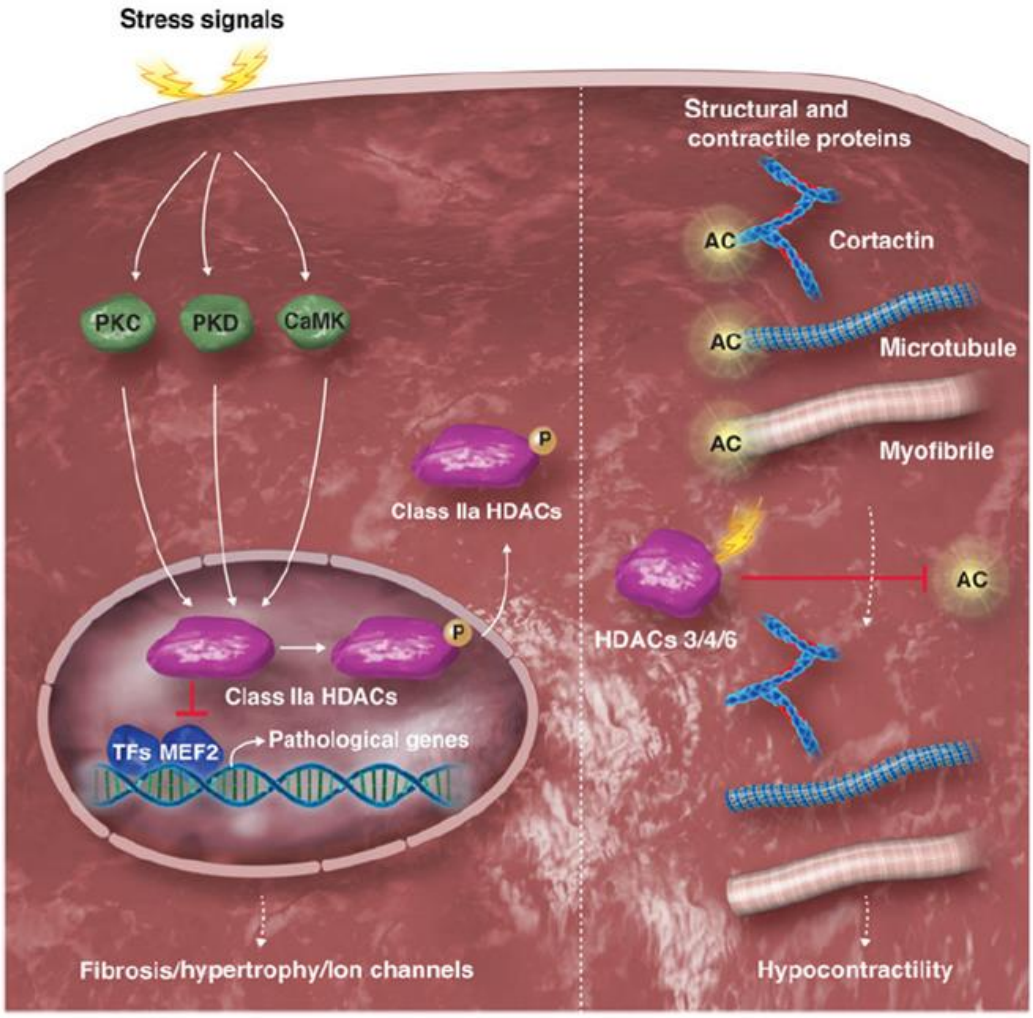

Figure 3: Effects of cardiac stress signals on HDACs in proteostasis regulation. Class IIa HDACs normally repress pathological cardiac gene expression. In response to stress signals, kinases, including protein kinase C (PKC), protein kinase D (PKD) and calcium/calmodulin-dependent kinase (CaMK), directly phosphorylate class IIa HDACs to trigger their nuclear export. The nuclear export of class IIa HDACs triggers derepression of downstream target pathological genes and thereby contributes to structural remodeling such as fibrosis, hypertrophy and changes of ion channel expression. In the cytoplasm, cardiac stress activates HDAC6, HDAC3 and HDAC4. HDAC6 deacetylates substrates such as α-tubulin and cortactin, resulting in degradation of structural proteins and functional loss. In addition, during stress, HDAC3[27], HDAC4[26] and HDAC6[28] colocalize with sarcomeric proteins and contribute to hypocontractility by deacetylation of sarcomeric and cytosketal proteins.

3.2 Role of HDACs in transcriptional reprogramming

The involvement of HDACs in AF is recently recognized. Two studies indicate that HDAC modulate transcriptional reprogramming in AF, possibly via epigenetic regulation. Liu et al.[24] were the first to show that HDAC inhibition in cardiac hypertrophy protects homeo-domain-only protein (HopX) transgenic mice from atrial arrhythmia inducibility and fibrosis.[24] In this study, HopX mice, which recruit HDAC to the chromatin to induce SRF-dependent transcription and cardiac hypertrophy, were either treated or untreated with the pan-HDAC inhibitor trichostatin A (TSA) and compared to control groups. TSA treated mice were protected against tachypacing-induced atrial arrhythmias and fibrosis formation. Moreover, this study suggests AF to originate from epigenetic regulation, since these mice feature increased HDAC activity, recruited by overexpressing the HopX. In addition, Lkhagva et al.

recently found that HDAC inhibition by the pan-HDAC inhibitor TSA and class I HDAC inhibitor MPT0E014, reduces arrhythmogenesis and AF inducibility by reducing calcium sparks via normalization of NCX1 and ryanodine receptor expression in rabbit pulmonary vein cardiomyocytes.[55] Also, HDACs may potentially modulate AF-induced activation of the fetal gene program via the chromatin-remodeling protein, Brg1. Brg1 preserves fetal cardiac differentiation by interacting with HDAC and poly (ADP ribose) polymerase (PARP), thereby repressing α-MHC and activating β-MHC expression.[77] Brg1 expression is turned off in adult cardiomyocytes and becomes reactivated during cardiac stress. Upon reactivation, Brg1 forms a complex with HDAC and PARP, in turn resulting in the α-MHC to β-MHC shift. Treatment of SW13 cells with TSA protects the cells from Brg1-induced shift of α-MHC to β-MHC.[77] Thus the apparent role of HDACs in AF warrants the exploration of the modulating role of HDACs in transcriptional reprogramming in AF-induced structural and functional cardiomyocyte remodeling.

3.3 HDAC modulate contractile function via PTM

Beyond altering chromatin structure and gene expression, HDAC also affect contractility by deacetylating contractile and structural proteins.[6, 26-28] We recently identified a specific class IIb HDAC, HDAC6, to derail proteostasis via PTM of structural proteins, resulting in structural remodeling and AF promotion.[6] Specifically, tachypacing increases HDAC6 expression and activity, resulting in deacetylation of α-tubulin in HL-1 cardiomyocytes and dog atrial cardiomyocytes. In turn, deacetylated α-tubulin is degraded by calpain, causing disruption of the microtubule structure and consequently contractile dysfunction.[6] Importantly, comparable findings were obtained in patients with persistent AF, suggesting HDAC6-induced PTM via deacetylation and degradation of α-tubulin to represent a common feature of remodeling in both experimental and clinical AF. In addition to α-tubulin, also cortactin and HSP90 are reported as substrates of HDAC6.[78, 79] Acetylated cortactin promotes polymerization of the actin cytoskeleton and regulates the structure of cardiomyocytes, actions that are counteracted by HDAC6 mediated deacetylation of cortactin. Under physiological conditions, HDAC6 complexes with HSP90 and heat shock factor-1 (HSF-1), thereby suppressing the heat shock response. Cardiac stress can induce the ubiquitination of proteins, which bind to the ubiquitin binding site of HDAC6, resulting in the dissociation of the complex. In turn, HSF-1 can translocate to the nucleus to induce the heat shock response, a response we previously found cardioprotective in AF.[23, 80] Consequently, this action is independent of the deacetylation activity of HDAC6 and it is still unknown whether it plays a role in AF.

3.4 HDACs and protein degradation

As mentioned, AF induced HDAC6 activation initiates deacetylation of α-tubulin thus promoting a shift from the polymerized microtubule structure into its depolymerized form, thereby causing calcium transient loss and contractile dysfunction. Deacetylated and depolymerized α-tubulin is susceptible to accelerated degradation by the protease calpain.[6] Also in patients with permanent AF, activation of HDAC6 deacetylates and degradates α-tubulin, and an inverse correlation with calpain activity is described. These results are consistent with previous reports demonstrating that microtubule network disruption causes changes in calcium signaling of cardiomyocytes, including reduction of contractions,[81] L-type calcium current[82] and calcium transient amplitude,[83] which are known to

underlie AF progression.[5] Besides, the involvement of calpain is in accord with observations that calpain is strongly activated during experimental and clinical AF[16, 18, 21] and with data showing degradation of brain α-tubulin by calpain.[84]

3.5 Beyond AF – derailment of proteostasis and HDAC activation in other cardiac diseases

So far, HDAC6 was found to play a key role in experimental and clinical AF progression by derailment of cardiomyocyte proteostasis.[6] In addition to its role in AF progression, HDAC6 is also involved in other cardiovascular diseases. Mckinsey and colleagues observed that the catalytic activity of HDAC6 is consistently increased in myocardium subjected to hypertensive stimuli, but not during physiologic hypertrophy.[50] The HDAC6 catalytic activity is also induced by diverse extracellular stimuli, including norepinephrine and phenylephrine in cultured cardiomyocytes and fibroblasts.[50] Recently, they found that both HDAC6 null mice and mice treated with the HDAC6 inhibitor, tubastatin A, are protected against systolic dysfunction caused by angiotensin II. HDAC6 null mice also exhibited improved left ventricular function in the setting of pressure overload mediated by transverse aortic constriction.[28] HDAC6 inhibition appeared to preserve systolic function, in part, by enhancing myofibrillar force generation.[28] Furthermore, a very recent study showed that prevention of tubulin deacetylation by HDAC6 inhibition was protective in a mouse model of proteinopathy-induced heart failure.[36] Inhibiting α-tubulin deacetylation, by utilizing the FDA-approved drug suberoylanilide hydroxamic acid (SAHA), reduced protein aggregates in cardiomyocytes and resulted in a substantial improvement in cardiac function. Mechanistically, the inhibition of HDAC6 increases autophagy in cardiomyocytes, which can also be accomplished by voluntary exercise.[36] Taken together, next to the findings in AF, additional studies define novel roles for HDAC6 in various cardiac diseases and indicate the general therapeutic potential of HDAC6 inhibitors to treat cardiac dysfunction in patients.

4.Therapeutic implication for AF: HDAC6 as therapeutic target in AF

As we discussed above, derailment of proteostasis is involved in AF initiation and progression and HDAC6 represents an important modulator in cardiac proteostasis. Promoting maintenance of proteostasis by HDAC6 inhibition may prevent structural remodeling in cardiomyocytes and thereby attenuate AF progression.

Pan-HDAC inhibitors and class I HDAC inhibitors have been studied in various cardiac diseases and consistently reduced pathological cardiac remodeling, including heart failure.[6, 6, 24, 25, 36, 50, 55, 64] In terms of potential toxicity and side effects, pan-HDAC inhibitors are generally regarded as effective and well tolerated for the treatment of cancer.[85] Reported side effects include nausea, fatigue, transient thrombocytopenia and, in some instances, myelosuppression.[86-89] Since cancer therapy is frequently based on a maximum tolerated dose, the efficacious dose of pan-HDAC inhibitors in AF may be significantly lower and hence well tolerated. Among the HDACs, HDAC6 emerges as a key regulator in AF progression and therefore represents an interesting druggable target. Inhibition of HDAC6 by tubacin protects against AF-induced de-acetylation and subsequent depolymerization and degradation of α-tubulin by calpain.[6] In this way, tubacin conserves α-tubulin proteostasis, and contractile function in experimental cardiomyocyte and *Drosophila* model systems for AF.[6] However,

tubacin is not suitable for *in vivo* studies, because of its high lipophilicity (LogP = 6.36) and complicated synthesis.[90] Other HDAC6 inhibitors, such as tubastatin A and ricolinostat (ACY-1215) have been developed, and have shown beneficial effects in mouse models for neurodegenerative diseases and cancer.[90-92] Recently, the first evidence was provided for the efficacy of tubastatin A in the dog model for AF.[6] Dogs treated with tubastatin A were protected against atrial tachypacing-induced derailment of α-tubulin homeostasis and electrical remodeling, resulting in improved cellular Ca^{2+} handling and contractile function, effectively counteracting AF progression. These *in vivo* findings strengthen the notion that HDAC6 inhibitors represent a novel therapeutic approach in AF. Trials with tubastatin A or ricolinostat should be initiated to determine the clinical potential of HDAC6 inhibitors in the treatment of first onset AF and the prevention of post-surgery AF. Interestingly, ricolinostat is currently tested in phase I and II clinical trials for the treatment of multiple myeloma, and so far, no serious side effects have been reported, making it an interesting candidate drug to test in patients with AF.[93]

In summary, the proper function of cardiomyocytes depends critically on the maintenance of a healthy proteostasis. Impairment of proteostasis has a prominent role in the pathophysiology of common human cardiac diseases including AF. So far, HDAC6 has been recognized as key modulator controlling cardiac proteostasis by changing acetylation status of structural proteins, leading to changes in protein function and the modulation of their degradation. Therefore, inhibitors of HDAC6 may represent a novel therapeutic target to treat clinical AF.

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